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Research Article

Isotopic Abundance Ratio Analysis and Structural Characterization of the Consciousness Energy of Healing Treated Magnesium Gluconate

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Abstract

Magnesium gluconate is the best source of magnesium for the human body, which is required for the metabolic processes, normal functioning of nerves, cells, muscles, bones, heart, etc. The aim of this research was to investigate the impact of the Trivedi Effect® on Mg-gluconate on the structural properties and isotopic abundance ratio using LC-MS and NMR spectroscopy. Magnesium gluconate sample was divided into two parts – one part was control sample, and another part was received the Biofield Energy Healing Treatment by a famous Biofield Energy Healer, Mr. Mahendra Kumar Trivedi is known as treated sample. The LC-MS of both the samples revealed the presence of protonated magnesium gluconate mass $[M+H]^+$ peak at m/z 415 (calcd for $C_{12}H_{23}MgO_{14}^+$, 415.09) at the retention time of 1.68 minutes. However, the relative peak intensities of the Biofield Energy Treated sample were significantly altered compared with the control sample. The isotopic abundance ratio of P_{M+2}/P_M ($^{26}Mg/^{24}Mg$ or $^{18}O/^{16}O$) was significantly increased by 58%, in the treated sample compared with the control sample. Briefly, ^{18}O and ^{26}Mg contributions from $C_{12}H_{23}MgO_{14}^+$ to m/z 417 in the Biofield Energy Treated magnesium gluconate was significantly increased compared with the control sample. The proton and carbon signals for OH, CH, CH_2 and CO groups in the proton and carbon NMR spectra of the Biofield Energy Treated sample were almost similar compared to the control sample. The improvement in the isotopic abundance ratios of the treated magnesium gluconate might be due to the possible mediation of neutrinos via the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the treated magnesium gluconate might improve strong atomic bond, increase the stability, and alter the rate of metabolic reactions in the body. Thus, the Biofield Energy Treated magnesium gluconate would be supportive to design the novel potent enzyme inhibitors using its kinetic isotope effects. Similarly, the Biofield Energy Treated magnesium gluconate could be valuable for designing better pharmaceutical and nutraceutical formulations, which might provide better therapeutic response against various diseases such as diabetes mellitus, aging, allergy, immunological disorders, inflammatory diseases, and other chronic infections.

Introduction

Magnesium gluconate (Mg-Gluconate; $C_{12}H_{22}MgO_{14}$) is the organometallic compound of magnesium with gluconic acid [1]. The bioavailability of magnesium gluconate was experimentally proved to be highest, and it is the best salt among other magnesium salts like acetate, aspartate, carbonate, chloride, citrate, lactate, sulfate, etc. which is physiologically acceptable [2,3]. It is the best source of magnesium for the human body. Magnesium is one of the most abundant mineral in the human body, which is required for more than 300 metabolic processes, normal functioning of nerves, cells, muscles, bones, heart, etc. [4]. Magnesium gluconate is a powerful antioxidant, and it is useful for the prevention and treatment of many diseases such as allergies, Alzheimer's disease, asthma, cardiovascular diseases, cancer, diabetes, inflammatory diseases, immunological disorders, pre-eclampsia, eclampsia, oxidative stress induced ischemia/reperfusion injury, etc. [4-8]. It is also used for the neuroprotection [9] and labor in women as an oral tocolytic agent [10]. Certain situations such as poor diet, severe diarrhea/vomiting, problem in stomach/intestinal absorption, diabetes, and use of drugs such as furosemide and hydrochlorothiazide, etc. reduce the magnesium quantities in the body [12]. Magnesium deficiency can be fulfilled by a well-balanced diet and magnesium supplements [13]. Therefore, Mg-Gluconate was contributed as one of the important components in the formulation as a source of Mg for the prevention and treatment of various human diseases. Physicochemical properties of nutraceutical compounds play an important role in its quality. Recently, the Biofield Energy Treatment proved scientifically altering the physicochemical properties of nutraceutical compounds [14-17].

The Biofield Energy (the Trivedi Effect®) is an infinite, dynamic, and paradimensional electromagnetic field exists surrounding the human body, resulting the continuous emission of low-

level light, heat, and acoustical energy from the body, which can freely flow between the human and environment [18,19]. There are several Biofield Energy Healing Therapies that are known for their significant impacts against various disease conditions [20]. Biofield Energy Healers possess the ability to harness the energy from the "Universal Energy Field" and can transmit this energy into any living or non-living object(s). The objects receive the Biofield Energy Healing Therapy respond into useful way and process is called Biofield Energy Healing Treatment. Such energy therapies are recommended by the National Institute of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM), and they included them under the Complementary and Alternative Medicine (CAM) due to their several advantages [21]. Biofield Energy Treatment has been drawn attention for its scientifically measurable ability to transform the characteristic properties of a wide variety of living and non-living objects [22-36]. The Trivedi Effect®-Biofield Energy Healing Treatment scientifically proved to have significant outcome in various fields such as, pharmaceuticals [24-26], material science [27,28], organic chemistry [29,30], microbiology [31,32], oncology [19,33], biotechnology [34,35], and agriculture [18,35,36]. In order to influence the better solubility and bioavailability of pharmaceutical/nutraceutical compounds the Trivedi Effect®-Energy of Consciousness Healing Treatment is a scientifically proven economical approach and well known for its significant impact on the physical, chemical, and structural properties of the drug molecule [20-28]. The scientific study indicated that the Trivedi Effect®-Biofield Energy Healing Treatment might be an alternate method for altering the natural isotopic abundance ratio of the substances through the possible mediation of neutrinos [37-39]. The natural stable isotope ratio analysis of material has wide applications for understanding the isotope effects due to the change in the isotopic composition [40,41]. The mass spectrometry techniques such as gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS), are widely applied for the analysis of isotope ratio with sufficient precision [42]. Hence in this study, the LC-MS and NMR (Nuclear Magnetic Resonance) techniques were used to characterize the structural properties of the magnesium gluconate. Subsequently, LC-MS based isotopic abundance ratio (P_{M+1}/P_M and P_{M+2}/P_M) of Mg-Glucoante samples was aimed to evaluate the influence of the Trivedi Effect®-Consciousness Energy Healing Treatment on the isotopic abundance ratio.

Materials and Methods

Chemicals and reagents

Magnesium gluconate was procured from Tokyo Chemical Industry Co., Ltd., Japan. All other chemicals which used in the experiment were of analytical grade available in India.

Consciousness energy healing treatment strategies

Magnesium gluconate, the test compound was divided into two parts. One portion of magnesium gluconate was denoted as the control sample, which did not receive the Biofield Energy Treatment. Besides, the other part of magnesium gluconate was termed as Biofield Energy Treated sample, which received the Energy of Consciousness Healing Treatment by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi remotely under the standard laboratory conditions for 3 minutes. On the other hand, the control magnesium gluconate has received treatment from a "sham" healer under the same laboratory conditions, where the "sham" healer did not have any knowledge about the Biofield Energy. Finally, both the samples of magnesium gluconate samples were kept in similar sealed conditions and further characterized by using LC-MS and NMR analytical techniques.

Characterization

Liquid Chromatography-Mass Spectrometry (LC-MS) and Isotopic Abundance Ratio Analysis: The LC-MS analysis of the Mg-gluconate was carried out using LC-Dionex Ultimate 3000 and MS-TSQ Endura (USA) equipped with a photo-diode array (PDA) detector connected with a triple-stage quadrupole mass spectrometer (Thermo Scientific TSQ Endura, USA) with a Thermo Scientific Ion Max NG source and heated electrospray ionization (ESI) probe. The analysis was performed on a reversed-phase Zorbax SB-C18 100 X 4.6 mm, 3.5 µm column (column temperature 40˚C) in gradient mode (follow condition: 0.1 min-5%B, 5.0 min-5%B, 15.0 min-60%, 25.0 min-95%B, 35.0 min-95%B, 40.0 min-5%B and 45.0 min-5% B) in the liquid chromatography. The mobile phase was 2 mM ammonium formate and 0.5% formic acid in water (mobile phase A), and acetonitrile (mobile phase B) at a constant flow rate of 0.6 mL/min. 10 µL sample was injected with a total run time of 45 min. Peaks were monitored at 250 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode.

The natural abundance of each isotope (Mg, C, O, and H) can be anticipated from the isotopic peak intensity compared to the base peak. The values of the natural isotopic abundance of the common elements are obtained from the literature [43-46]. The isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and treated Mg-gluconate were calculated.

% change in isotopic abundance ratio = $[(IAR_{Treated} - IAR_{Control})/IAR_{Control})] \times 100$

Where IAR: isotopic abundance ratio in the control and treated sample.

Nuclear magnetic resonance (NMR) analysis

^1H NMR was recorded at 400 MHz on Agilent-MRDD2 FT-NMR spectrometer at room temperature. Approximately 3 mg of the sample was dissolved in DMSO-d_6. Chemical shifts (d) were in parts per million (ppm) related to the solvent's residual proton chemical shift {$(CD_3)_2SO$, $\delta = 2.5$}. Similarly, ^{13}C NMR was measured at 100 MHz on Agilent-MRDD2 FT-NMR spectrometer at room temperature. Approximately 25 mg of the sample was dissolved in DMSO-d_6. Chemical shifts (d) were in parts per million (ppm) related to the solvent's residual carbon chemical shift {$(CD_3)_2SO$, $\delta = 39.52$}.

Table 1: Isotopic abundance ratio analysis of control and treated Mg-gluconate.

Parameter	Control sample	Biofield Energy Treated sample
P_M at m/z 415 (%)	100	100
P_{M+1} at m/z 416 (%)	26.34	26.34
P_{M+1}/P_M	0.2634	0.2634
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		0.000
P_{M+2} at m/z 417 (%)	7.38	11.66
P_{M+2}/P_M	0.0738	0.1166
% Change of isotopic abundance ratio (P_{M+2}/P_M) with compared to the control sample		58.00

Results and Discussion

Liquid chromatography-mass spectrometry (LC-MS) analysis

The chromatograms of both the control and the Trivedi Effect®-Biofield Energy Treated magnesium gluconate (Figure 1) showed two sharp peaks near the retention time (R_t) of 1.53 and 1.68 minutes. The % peak area of the treated sample was found to be very close to the control sample (Figure 1). This indicated that the polarity of the Biofield Energy Treated sample remained unaltered compared with the control sample of magnesium gluconate.

The ESI-MS (+ve mode) spectrum of the control sample of magnesium gluconate at R_t of 1.53 minutes (Figure 1) exhibited the presence of the mass of gluconate ion adduct with one magnesium ion and two sodium ion at *m/z* 265 $[M+Mg+2Na]^+$ (calcd for $C_6H_{11}MgNa_2O_7^+$, 265.01) along with other mass fragmentation peak at lower *m/z* 242 $[M+Mg+Na]^+$ (calcd for $C_6H_{11}MgNaO_7^+$, 242.02), *m/z* 219 $[M+Mg+Na]^+$ (calcd for $C_6H_{11}MgO_7^+$, 219.03), etc (Figures 2 and 4). Monoisotopic adducts formation, either with solvents, alkali or other metal ions or with other contaminating components, is frequently observed in ESI analysis [46,47]. The base peak was observed in the mass spectrum at *m/z* 118 (calcd for $C_4H_7O_4^+$, 118.03) in the control sample. A similar type of mass fragmentation peaks was observed in the Biofield Energy Treated magnesium gluconate.

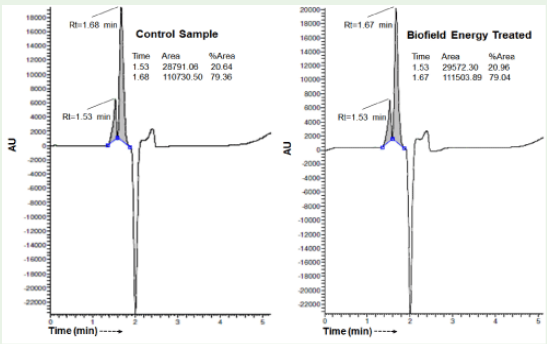

Figure 1: Total ion chromatograms of the control and treated Mg-gluconate.

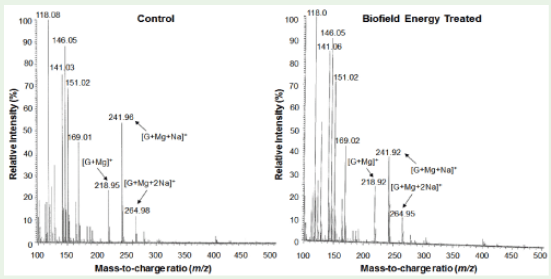

Figure 2: The mass spectra of the control and treated Mg-gluconate at R_t 1.53 minutes in the chromatograms. G: Gluconate; Mg: Magnesium; Na: Sodium.

Similarly, mass spectra of the control and Biofield Energy Treated samples at R_t of 1.68 minutes (Figure 3) exhibited the presence of the molecular mass of magnesium gluconate adduct with hydrogen ion at *m/z* 415 (calcd for $C_{12}H_{23}MgO_{14}^+$, 415.09). Many typical fragment ion peaks in the lower *m/z* region of the protonated magnesium gluconate ion $[M+H]^+$ (*m/z* 415) were observed in both the control and Biofield Energy Treated samples (Figures 3 and 4) at *m/z* 397 (calcd for $C_{12}H_{21}MgO_{13}^+$, 397.08), 317 (calcd for $C_{10}H_{13}MgO_{10}^+$, 317.04), 265 (calcd for $C_6H_{13}MgO_9^+$, 265.04 or $C_6H_{11}MgNa_2O_7^+$, 265.01), 219 (calcd for $C_6H_{11}MgO_7^+$, 219.03), and 118 (calcd for $C_4H_6O_4^{\cdot}$, 118.03).

The mass spectra of the both the samples (Figure 4) displayed almost similar type of fragmentation pattern. The molecular ion peak at *m/z* 415 corresponding to $C_{12}H_{23}MgO_{14}^+$ exhibited 100% relative peak intensity in both the mass spectra (Figure 3). The relative peak intensities of the other ion peaks in the Biofield Energy Treated sample were significantly changed compared with the control sample.

Isotopic Abundance Ratio Analysis

The mass spectra of both the Mg-gluconate samples showed the mass of a protonated molecular ion at *m/z* 415 ($C_{12}H_{23}MgO_{14}^+$) with 100% relative intensities. The theoretical calculation of isotopic peak P_{M+1} for the protonated magnesium gluconate presented as below:

P (^{13}C) = [(12 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 13.20%

P (^{2}H) = [(23 x 0.015%) x 100%] / 100%= 0.35%

P (^{25}Mg) = [(1 x 12.66%) x 100%] / 100% = 12.66%

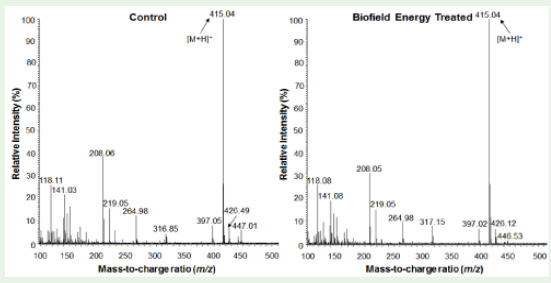

Figure 3: The mass spectra of the control and treated Mg-gluconate at R_t 1.68 minutes in the chromatograms.

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Table 2: ^{1}H and ^{13}C NMR data of the control and treated Mg-gluconate.

Position	^{1}H NMR δ (ppm)			^{13}C NMR δ (ppm)	
	Number	Control	Treated	Control	Treated
1, 1'	4H (CH_2) & 2H(OH)	3.93 (d, *J* = 10 Hz) & 6.38 (br, s)	3.91 (dd, *J* =22 Hz), 6.31 (br, s)	63.38	63.38
2, 2'	2H (CH) & 2H(OH)	3.54 (br, t, *J* = 29.2 Hz) & 4.56 (s)	3.55 (br, t, *J* = 22.8 Hz) & 4.55 (s)	70.25	70.25
3, 3'	2H (CH) & 2H(OH)	3.54 (br, t, *J* = 29.2 Hz) & 5.13 (br, d)	3.55 (br, t, *J* = 22.8 Hz) & 5.12 (br, d)	71.01	71.01
4, 4'	2H (CH) & 2H(OH)	3.54 (br, t, *J* = 29.2 Hz) & 5.13 (br, d)	3.55 (br, t, *J* = 22.8 Hz) & 5.12 (br, d)	71.12	71.12
5, 5'	2H (CH) & 2H(OH)	3.41 (d, *J* = 15 Hz) & 4.32 (br, s)	3.41 (d, *J* = 35 Hz) & 4.32 (br, s)	72.64	72.62
6, 6'	--	--	--	175.28	175.18

br- broad, s- singlet, t- triplet, and d-doublet.

P (^{17}O) = [(14 x 0.04%) x 100%] / 100% = 0.56%

P_{M+1} *i.e.* ^{13}C, ^{2}H, ^{17}O, and ^{25}Mg assistances from $C_{12}H_{23}MgO_{14}^{+}$ to *m/z* 416 = 26.77%

Similarly, the theoretical calculation of isotopic peak P_{M+2} for the protonated magnesium gluconate presented as below:

P (^{26}Mg) = [(1 x 13.94%) x 100%] / 100% = 13.94%

P (^{18}O) = [(14 x 0.20%) x 100%] / 100% = 2.8%

P_{M+2} *i. e.* ^{18}O, and ^{26}Mg assistances from $C_{12}H_{23}MgO_{14}^{+}$ to *m/z* 416 = 16.74%

The calculated isotopic abundance of P_{M+1} value 26.77% was very close to the observed value 26.34% (Table 1). The probability of *A* + 1 and *A* + 2 elements having an isotope with one and two mass unit, respectively heavier than the most abundant isotope (*e. g.* ^{13}C, ^{2}H, ^{17}O, ^{25}Mg, ^{18}O, and ^{26}Mg) contributions to the mass of the isotopic molecular ion $[M+1]^{+}$ and $[M+2]^{+}$. Hydrogen did not contribute much to any isotopic *m/z* ratios because of less natural abundance compared to the natural abundances of carbon, magnesium and oxygen isotopes [45,49]. The calculations indicated that ^{13}C, ^{18}O, ^{25}Mg, and ^{26}Mg have the major contributions from magnesium gluconate to the isotopic mass peak at *m/z* 416 and 417.

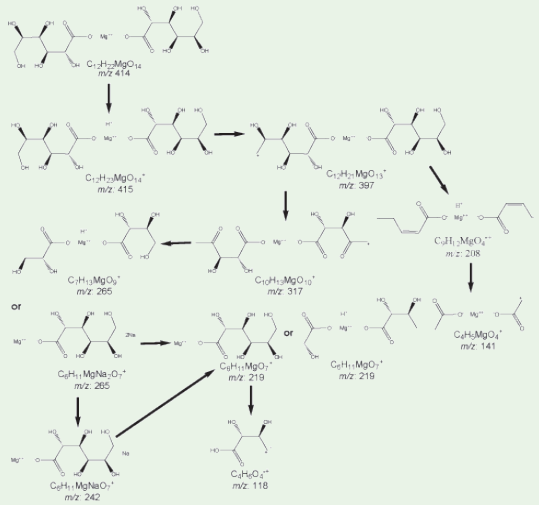

Figure 4: Proposed fragmentation pathway of Mg-gluconate.

Both the mass spectra of the control and treated samples indicated the presence of the protonated magnesium gluconate at *m/z* 415. Hence, P_M, P_{M+1}, and P_{M+2} of the magnesium gluconate at *m/z* 415, 416, and 417 of both the samples were obtained from the observed relative peak intensities of M^{+}, $[M+1]^{+}$, and $[M+2]^{+}$ peaks, respectively (Table 1).

P_M, P_{M+1}, P_{M+2} = the relative peak intensity of the parent molecular ion peak $[M^{+}]$, isotopic molecular ion peak $[M+1]^{+}$ and $[M+2]^{+}$, respectively

The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated sample was remained unaltered compared with the control sample (Table 1). Thus, ^{2}H, ^{17}O, ^{13}C, and ^{25}Mg contributions from $C_{12}H_{23}MgO_{14}^{+}$ to *m/z* 416 in the treated sample would be the same compared with the control sample. But, the isotopic abundance ratio of P_{M+2}/P_M in the treated sample was significantly improved by 58% compared with the control sample (Table 1). Thus, ^{18}O and ^{26}Mg contributions from $C_{12}H_{23}MgO_{14}^{+}$ to *m/z* 417 in the Biofield Energy Treated sample significantly increased compared with the control sample.

Increased or decreased isotopic abundance of $[M+1]^{+}$ and $[M+2]^{+}$ ions in the mass spectroscopic analysis of the several organic compounds suggested the change in the number of neutrons in the molecule [21,37-39]. It was then assumed that the alterations in atomic mass and the atomic charge could be through the possible mediation of neutrinos oscillation. The improvement in the isotopic abundance ratios of the treated magnesium gluconate might be due to the possible mediation of neutrinos *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the treated magnesium gluconate might have improved strong atomic bond, increase the stability, and alter the rate of metabolic reactions in the body [50]. Thus, it is expected that Energy of Consciousness Healing Treatment might provide the necessary energy for the neutrino oscillations. The modification of neutrinos inside the molecule, in turn, modified the fundamental properties like particle size, density, chemical reactivity, thermal behavior, binding energy, selectivity, etc. of a compound [21,37,38].

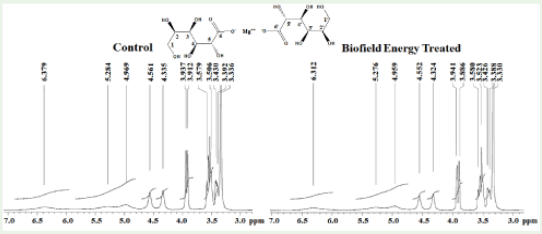

Figure 5: The ^{1}H NMR spectra of the control and treated Mg-gluconate.

As a result, kinetic isotope effect was altered with the variation in the isotopic abundance ratio of one of the atoms in the reactants in a chemical reaction, which is very useful to study the reaction mechanism in addition to understanding the enzymatic transition state and all aspects of enzyme mechanism that is supportive for designing enormously effective and specific inhibitors [21,37,38,42]. As Mg is an essential cofactor for various enzymatic reactions, the treated Mg-gluconate that had altered isotopic abundance ratio (P_{M+2}/P_{M}) might be advantageous for the study of enzyme mechanism as well as supports in the designing of novel potent enzyme activators/inhibitors.

Nuclear magnetic resonance (NMR) analysis

The ^{1}H and ^{13}C NMR spectra of the control and treated Mg-gluconate are shown in Figures 5 and 6, respectively. NMR assignments of the control and treated Mg-gluconate are presented in Table 2. The signals for the protons coupling of CH_2, CH, and OH protons in the ^{1}H NMR spectra (Figure 5) of magnesium gluconate were observed in the control and Biofield Energy Treated samples in the range of δ 3.4 to 6.3 ppm (Table 2), which was almost similar to the reported proton spectrum of sodium gluconate [21,51]. Similarly, the carbon signals for CO, CH_2 and CH groups in the ^{13}C NMR spectrum (Figure 6) of the Biofield Energy Treated sample were almost similar compared with the control sample (Table 2). Therefore, it is confirmed that the structure of the magnesium gluconate in the Biofield Energy Treated sample remained the same as compared to the control sample.

Conclusions

The current research work evaluated the structural characterization of magnesium gluconate using LC-MS and NMR techniques and the influence of the Trivedi Effect® - Consciousness Energy Healing Treatment on the isotopic abundance ratio. The LC-MS of both the samples revealed the presence of protonated magnesium gluconate mass $[M+H]^{+}$ peak at *m/z* 415 (calcd for $C_{12}H_{23}MgO_{14}^{+}$, 415.09) at the retention time of 1.68 minutes. The isotopic abundance ratio of P_{M+2}/P_{M} (^{18}O/^{16}O or ^{26}Mg/^{24}Mg) was significantly increased by 58%, in the Biofield Energy Treated sample compared with the control sample. Briefly, ^{18}O and ^{26}Mg contributions from $(C_{12}H_{23}MgO_{14})^{+}$ to *m/z* 417 in the Biofield Energy Treated sample was significantly increased compared with the control sample. The improvement in the isotopic abundance ratios of the treated magnesium gluconate might be due to the possible mediation of neutrinos *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the treated magnesium gluconate might have improved strong atomic bond, increase the stability, and alter the rate of metabolic reactions in the body. The Trivedi Effect®-Biofield Energy Healing Treated magnesium gluconate might exhibit isotope effects due to its increased isotopic abundance ratio compared with the control sample. Thus, the Biofield Energy Treated magnesium gluconate might be helpful to understand the enzymatic reactions as well as to designing the novel potent enzyme inhibitors by using its kinetic isotope effects. Besides, the Trivedi Effect®-Energy of Consciousness Healing Treatment could be a useful and economical approach in the design of more efficacious nutraceutical and/or pharmaceutical formulations against septic shock, arrhythmias, diabetes mellitus, asthma, cancer, allergies, inflammatory diseases, immunological disorders, gestational hypertension, etc.

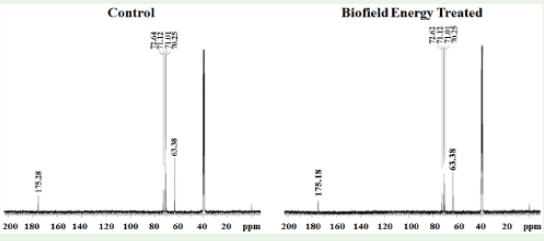

Figure 6: The ^{13}C NMR spectra of the control and treated Mg-gluconate.

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